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First Report of Carbapenem-Resistant *Acinetobacter nosocomialis* Isolates Harboring IS*Aba*1-bla_{OXA-23} Genes in Latin America

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In recent years, different resistance genes have been found in *Acinetobacter* spp., especially in the species *A. baumannii*. We describe two isolates of carbapenem-resistant *A. nosocomialis* harboring IS*Aba*1-bla_{OXA-23} and bla_{OXA-51} found in patients from the city of Porto Alegre, southern Brazil. To the best of the authors' knowledge, this is the first report of carbapenem-resistant *A. nosocomialis* in Latin America.

In recent years, *Acinetobacter* spp. have been described as important pathogens in outbreaks of nosocomial infection worldwide, especially in intensive care units (1). In particular, the species *A. baumannii* has presented an increased rate of antimicrobial resistance (2, 3). Carbapenems, once regarded as the treatment of choice for infections caused by *Acinetobacter* spp., are no longer effective in some cases (2). The main mechanism of carbapenem resistance among *Acinetobacter* spp. is the production of β-lactamases, in particular class D β-lactamases (oxacillinases), associated with promoter gene sequence IS*Aba*1 (3). Among oxacillinases, the most prevalent one is bla_{OXA-23}, identified in mobile genetic elements. Chromosomally located bla_{OXA-51} genes, in turn, do not always confer carbapenem resistance but are used to identify *A. baumannii*, as it is believed to be intrinsic to this species (4–6).

Traditionally, the bla_{OXA-23} and bla_{OXA-51} genes are associated with *A. baumannii* only, but recently some authors have described the presence of such genes in non-*A. baumannii* species. The bla_{OXA-23} gene was found in *A. pittii* (*Acinetobacter* genomic species 3) in the Irish Republic in 2006 and in *A. nosocomialis* (*Acinetobacter* genomic species 13TU) in South Korea and Thailand in 2012 (7, 8). Moreover, bla_{OXA-51} preceded by IS*Aba*1 has been found in carbapenem-resistant *A. nosocomialis* in Taiwan (9).

In this study, we evaluated a set of non-*A. baumannii* species and found two isolates of carbapenem-resistant *A. nosocomialis* with the IS*Aba*1-bla_{OXA-23} and bla_{OXA-51} genes, obtained from patients living in the city of Porto Alegre, southern Brazil.

A total of 118 isolates were evaluated, obtained over the year 2011 from clinical specimens of *Acinetobacter* spp. previously identified using conventional methods. Isolates were identified to the species level using *gyrB* multiplex PCR as described by Higgins et al., with few modifications (10). Briefly, we used seven primers at a total reaction volume of 25 μl, consisting of 0.2 μM each primer, 1.5 mM $MgCl_2$, 1× 0.2 mM each deoxynucleoside triphosphate (dNTP), and 1 U *Taq* DNA polymerase. The PCR program consisted of initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation (94°C for 1 min), annealing (56°C for 30 s), and extension (72°C for 1 min), with a final extension step at 72°C for 10 min. Species identification was also evaluated by PCR with primers targeting the 16S-23S rRNA intergenic transcribed spacer (ITS) region, followed by sequence analysis (11). Oxacillinase genes (bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, bla_{OXA-58}, and $bla_{OXA-143}$) were identified using multiplex PCR with specific primers. Isolates testing positive for oxacillinase genes were subjected to a PCR program for the promoter sequence IS*Aba*1 (10, 12, 13).

Imipenem and meropenem MICs were determined in duplicate using the Clinical and Laboratory Standards Institute broth microdilution method (14). *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212 were used as controls.

A total of 106 (89.8%) isolates proved to be *A. baumannii*. Twelve non-*A. baumannii* isolates were identified, including 6 (5.1%) *A. nosocomialis* isolates, 5 (4.2%) *A. pittii* isolates, and 1 (0.8%) *Acinetobacter* genomic species 10 isolate, with 100% concordance to species of the *A. baumannii-A. calcoaceticus* complex by two PCR methods tested. The bla_{OXA-51} and bla_{OXA-23} genes were identified in 5 (4.3%) and 4 (3.4%) non-*A. baumannii* isolates, respectively. Of the five isolates that tested positive for bla_{OXA-51}, four were *A. nosocomialis* and one was *A. pittii*. Among the four isolates positive for bla_{OXA-23}, three were *A. nosocomialis* and one was *A. pittii*. No other oxacillinases were found. For the first time in Latin America, IS*Aba*1 upstream of the bla_{OXA-51} and bla_{OXA-23} genes was identified in two isolates of carbapenem-resistant *A. nosocomialis* (Table 1). The presence of oxacillinase genes in non-*A. baumannii* isolates had already been described in studies from China, South Korea, and Singapore, which underscores the potential clinical significance of these species (7–9, 15).

It is worthy of note that two isolates of carbapenem-susceptible *A. nosocomialis* and one of *A. pittii* were found to harbor bla_{OXA-23}. Notwithstanding, these isolates did not present IS*Aba*1 upstream of the oxacillinase genes. It is well established that the promoting sequence IS*Aba*1 has to be present to ensure oxacillinase expression and, consequently, the development of resistance. We also found that resistance to carbapenems was not necessarily related

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TABLE 1 Characteristics of non-*Acinetobacter baumannii* isolates

Acinetobacter species[a]	PCR result[b]				MIC (μg/ml)[c]	
	bla_{OXA-23}	bla_{OXA-51}	IS*Aba*1 upstream bla_{OXA-23}	IS*Aba*1 upstream bla_{OXA-51}	Imipenem	Meropenem
A. pittii[d]	−	−	−	−	≤0.5	≤0.5
A. nosocomialis[e]	−	−	−	−	≤0.5	≤0.5
A. pittii	−	−	−	−	≥256	64
A. pittii	−	−	−	−	≤0.5	≤0.5
Acinetobacter genospecies 10	−	−	−	−	1	2
A. nosocomialis	+	+	+	+	128	64
A. nosocomialis	+	+	+	+	64	64
A. nosocomialis	−	−	−	−	64	64
A. pittii	+	+	−	−	≤0.5	≤0.5
A. nosocomialis	+	−	−	−	1	≤0.5
A. nosocomialis	+	+	−	−	≤0.5	≤0.5
A. pittii	−	−	−	−	≤0.5	≤0.5

[a] All isolates were identified using *gyrB* multiplex PCR and confirmed by 16S-23S intergenic transcribed spacer sequence analysis.
[b] +, positive; −, negative.
[c] MIC breakpoints for two carbapenems according to the Clinical and Laboratory Standards Institute broth microdilution method: resistant, ≥16 μg/ml; intermediate, 8 μg/ml; and susceptible, ≤4 μg/ml.
[d] Formerly *Acinetobacter* genomic species 3.
[e] Formerly *Acinetobacter* genomic species 13TU.

to oxacillinase genes, as one *A. nosocomialis* isolate and one *A. pittii* isolate resistant to carbapenems did not present these genes. In fact, it has already been shown that carbapenem resistance may be mediated by other mechanisms, e.g., porin loss and hyperexpression of efflux pumps (2).

Several studies have identified a variety of oxacillinases in carbapenem-resistant *A. baumannii* isolates. The main oxacillinases described include bla_{OXA-51}, bla_{OXA-23}, and $bla_{OXA-143}$; bla_{OXA-51} is believed to be intrinsic to *A. baumannii*, whereas the two latter genes have been associated with carbapenem resistance (16–20).

In this study, we found two isolates of *A. nosocomialis* harboring the IS*Aba*1 upstream of bla_{OXA-23} and bla_{OXA-51}, which has proved to confer resistance to carbapenems. These findings reinforce the importance of species-level identification, as there may be horizontal transfer of oxacillinase genes among different species of the *Acinetobacter* genus, a phenomenon previously described by Poirel et al. (21). In fact, non-*A. baumannii* species cannot be considered homogeneously susceptible to carbapenems and may lead to an increased prevalence of nosocomial infections caused by carbapenem-resistant *Acinetobacter* spp.

To the best of our knowledge, this is the first study reporting the identification of oxacillinase genes in non-*A. baumannii* isolates in Latin America.

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